Remarks

Claims 1-7, 10, 34-40 are pending in this application. Claims 1-7, 10, 34, and 35-40 are rejected. Claims 8-9 and 11-33 have previously been cancelled.

Claims 6 and 39 are cancelled.

New claims 41-47 are presented for examination.

Independent claims 1, 34, 35, and 36 are each amended such that the lymph node sample is histopathologically negative.

1. Claim Rejections – 35 U.S.C. § 103

Claims 1-3, 5-7, 10, 34-36, and 38-40 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Hoon et al. (U.S. Patent 6,057,105; 5/2/00) in view of Scholl et al. (2/01, Cancer Research, 61:823-826).

Independent claims 1, 35, and 36 require that nucleic acid be isolated from a histopathologically negative sentinel lymph node (SLN). Independent claim 34 requires that nucleic acid be isolated from blood. mRNA transcripts encoded by GalNAcT and PAX3 marker genes are amplified and quantified. The November 4, 2009 Office Action (the Office Action) concedes that Hoon et al. is deficient with respect to the utilization of PAX3 marker genes. To remedy this deficiency, the Office Action relies on Scholl et al. as providing a disclosure for using PAX3 to detect metastatic melanoma.

Applicants respectfully traverse this rejection. The present invention is patentable over the combination of Hoon et al. and Scholl et al. for at least three reasons. Scholl et al. does

not disclose a method for detecting metastatic melanoma. The combination of markers in the claimed method of the present invention provides unexpected results with respect to detecting occult melanoma. In particular, the combination of markers provides an unexpectedly reduced incidence of false positive results. The Office Action does not correctly apply established Federal Circuit law regarding obviousness with respect to combinations.

As set forth in the accompanying Declaration by Dr. German Pihan, an expert in pathology and molecular biology from Beth Israel Deaconess Medical Center/Harvard Medical School, "the pending patent application includes a judicious combination of markers with a sufficiently sensitive and specific detection method that makes it possible to predict with certainty important aspects of the clinical behavior of melanoma tumors." (Pihan Declaration attached as Exhibit A, Par. 10). Moreover, Dr. Pihan explains in his declaration that Scholl et al. does not disclose a method for detecting metastatic melanoma as alleged by the Examiner.

a. The Office Action incorrectly characterizes Scholl et al.

The combination of Hoon et al. and Scholl et al. is improper because, contrary to the assertions of the Office Action, Scholl et al. does not disclose a method of detecting metastatic melanoma using PAX3. In reality, Scholl et al. does not disclose a method for detecting metastatic melanoma at all. The Declaration of Dr. Pihan verifies that one skilled in the art of pathology and molecular biology would not interpret Scholl et al as providing a disclosure for detecting metastatic melanoma, and in particular, occult metastatic melanoma:

> As one skilled in the art of pathology and molecular biology, I state that the Examiner's conclusion is not substantiated By the data presented in the *Scholl et al reference.* The *Scholl et al reference* **does not teach a method of detecting melanoma.**
>
> Pihan Declaration, Par. 7.

Moreover, Scholl et al. does not disclose that PAX3 is useful for detecting melanoma in histopathologically negative sentinel lymph nodes nor in a body fluid as set forth in the independent claims. The combination of Hoon et al. and Scholl et al. does not render the invention obvious because one skilled in the art "would not interpret the teachings of the *Scholl reference* as suggesting that the detection of PAX3 in histopathologically negative lymph nodes would be functional as a test for occult melanoma metastasis." (Pihan Declaration, Par. 7) Thus, Scholl et al. does not remedy the admitted deficiencies of Hoon et al.

Significantly, Scholl et al. does not perform any detection of occult disease. In general, occult tumors will not have the same mRNA expression patterns as overt metastasis. Dr. Pihan explains both of these shortcomings of Scholl et al. as follows:

> In the *Scholl reference,* only a fraction of tissue samples with **histologically evident metastatic melanoma** expressed the transcription factor mRNA PAX3. No sample of lymph node without histologically obvious metastases – and therefore possibly harboring histologically occult micrometastases, which is at the core of the claimed method in the pending patent application - was included or ever conducted in the *Scholl reference*.
>
> Pihan declaration, Par. 8 (emphasis added)

Even with respect to the detection of PAX3 in overt melanoma metastases, the data in Scholl et al. would not provide a sufficient impetus for one skilled in the art to believe that PAX3 would be useful in detecting occult metastases in histopathologically negative lymph nodes.

Scholl et al. states that the experiments therein "suggest that *in situ* hybridization of PAX3 on paraffin-embedded tissue may represent a novel means to identify melanoma cell lesions." However, the actual experiments described in Scholl et al. are directed to the detection of PAX3 in melanoma cell lines, cultured melanoma cells, and in overt tumors from patients at

various stages of disease. Each experiment was knowingly designed to contain melanoma cells. At the very least, scientific logic would require a method for detecting metastatic melanoma to demonstrate the ability to detect which samples contain cancer from a set of samples containing both cancer-free and cancer-containing samples. Scholl et al. does not present any experimental results for samples not containing melanoma, thereby making it impossible to assess a rate of false positive results. Any useful test must have a reasonably lower rate of false positives.

Although studies involving melanoma cell lines and cultured melanoma cells have their place in medical research, the behavior of *in vivo* cancer cells is typically very different. No medical professional or regulatory agency would allow treatment of a patient that was only based on such cell lines because of the associated lack of predictability. With respect to cultured cell lines, Hoon et al. explains:

> The culturing conditions can cause alterations in cell metabolisms, therefore primary melanocyte culture cells are not always true representative of "normal" *in vivo* cells.
>
> Hoon et al., col. 34, ll. 4 - 7

Similarly, the data of the present invention demonstrates that melanoma cell lines do not provide a realistic view of *in vivo* behavior:

> **All four of the mRNA markers were expressed in 100% of melanoma cell lines**. However, the mRNA copy number for individual markers vary in individual cell lines. **Also, the mRNA copy levels in histopathology positive PE SLNs are not expressed in 100% but vary in individual SLNs.** The results are due to not only the heterogeneity in individual tumor tissues but also the portion of micrometastases which have completely removed from the PE-block in some cases at the time of pathological examination (H&E and IHC), and also the quantity of the amount of mRNA for each sample.

Specification, p. 12, ll. 6 - 14 (emphasis added)

The data in the present invention clearly shows that the expression patterns of cultured melanoma cell lines are not predictive of the behavior of *in vivo* melanoma cells.

Accordingly, for at least these reasons, claims 1-3, 5-7, 10, 34-36, and 38-40 are patentable under 35 U.S.C. § 103(a) over Hoon et al. in view of Scholl et al.

b. <u>The Invention Provides Unexpected Results</u>

The method of the present invention provides significant advantages over the prior art with respect to accuracy, sensitivity, and specificity. In particular, the methods of the invention have a significantly reduced incidence of false positive results. Even if the Office Action's view of combining markers were correct with respect to identifying more patients with occult disease (which it is not), the low incidence of false positive results is totally unexpected. The combined quantification of MAGE-A3, GalNAcT, MART-1, and PAX3 transcripts is particularly useful in excluding false positive results. For a given population of patients, the number of false positives will accumulate as more markers are used. The Specification of the present application provides the following summary of the prior art with respect to false positive results:

> . . . the multi-marker panels of prior art, such as those of the U.S. Pat. No. 6,057,105, do not have a significant predictive power in **disease recurrence**. The marker panels of prior art that were created for frozen sections would not be predictive in PE samples. Earlier studies had at the most a twenty-month follow-up (Li, W., 2000) and could not evaluate the predictive power of markers for disease recurrence, which takes 3-8 years. **Additionally, many of the markers utilized in earlier MM RT-PCRs were producing false-positive results** (Li, W., 2000, U.S. Pat. No. 6,037,129).

Specification, p. 10, ll. 1 – 8 (emphasis added)

Table 2 from the Specification quantifies the low false positive results by providing the specificities for MART-1, MAGE-A3, GalNacT, PAX3, as 1.000, 1.000, 1.000, 0.923, respectively (a value of 1 means that there are no false positives). In contrast to the prior art, the Specification explains:

> **Thus, the methods of the present invention, unlike conventional methods do not produce false-positive results.** Only one normal lymph node expressed PAX3 mRNA, however, the copy number was one. The values in ROC curve analysis and the sensitivity and specificity of each marker were acceptable and feasible for detection of metastatic melanoma in PE specimens.
>
> Specification, p. 23, ll. 14 – 18 (emphasis added)

In addition to having a low incidence of false positive results over all, the quantitative nature of the present invention allows a clinician to make a decision whether or not a low copy number should be treated as a positive result.

The combination of PAX3 and GalNacT has additional unique advantages beyond just a random combination of melanoma markers and beyond using a single marker. Specifically, the incidences of PAX3 and GalNacT in histologically negative lymph nodes are each 17% as determined by the experiments set forth in the Specification. These numbers are relatively high when it is realized that they relate to lymph nodes that are deemed cancer free. Neither Scholl et al. nor Hoon et al. suggest this distribution. The frequency of MART-1 and MAGE-A3 expression in histopathologically negative lymph nodes also set forth in the Specification is instructive in showing the unique advantages of the PAX3 and GalNacT:

> On the other hand, MART-1, MAGE-A3, GalNAcT, and PAX3 marker expression were detected from 162 patients with histopathologically proven melanoma-free SLNs (no evidence of

> tumor cells in SLN by H&E staining or IHC) as follows: 10 (6%), 8 (5%), 27 (17%), 28 (17%), respectively.
>
> Specification, p. 26, ll. 5 - 9

This data suggests that a combination of GalNAcT and PAX3 detects occult melanoma in negative lymph nodes with a frequency from 17% to 34%. This value is higher than for many markers. For example, if a combination of only MART-1 and MAGE-A3 were used, the expected frequency of detecting melanoma in histopathologically negative lymph nodes is 6 % to 11%. The data of the Specification shows that the combination of GalNAcT and PAX3 is special in predicting occult metastasis. Neither Hoon et al. nor Scholl et al. suggest anything special about the combination of GalNAcT with PAX3.

Accordingly, for at least these reasons, claims 1-3, 5-7, 10, 34-36, and 38-40 are allowable under 35 U.S.C. § 103(a) over Hoon et al. in view of Scholl et al.

c. The Office Action does not Properly Apply Federal Circuit Law

The Office Action assigns a higher level of predictability to the field of the invention than is justified. The chemical and biological fields are generally considered to be unpredictable. Cancer, in particular, is known to be a very unpredictable and variable disease. If this were not the case, a cure for cancer would have been found long ago. The Office Action would lead one to believe that all combinations of melanoma markers are "created equal." However, the Office Action's analysis does not accurately reflect the state of the art at the time the present patent application was filed. In reality, as set forth below, the prior art is replete with articles showing that many combinations of markers are of marginal or no clinical utility or have no advantage over a single marker.

The Office Action implicitly concludes that it was established at the time the present application was filed that detection of occult melanoma using a combination of markers is better in all instances than using a single marker and that conclusions regarding markers in breast cancer may be imputed to melanoma. The Office Action makes both these conclusions in total disregard to the numerous prior art references that are of record showing that multi-marker melanoma analysis was not established at the filing date and that many combinations of markers are not clinically useful. The Office Action provides the following analysis with respect to using multiple markers:

> Further, the abstract of Hoon et al states "Methods using multiple markers provide increased sensitivity over existing methods" and Example XIII of Hoon et al states "None of the markers alone would have been able to detect cancer cells in all of the specimens. This variation in marker detection reflects the heterogeneity of tumor cells. In conclusion, multiple markers are more sensitive to detection of breast cancer than any one marker".

Interestingly, the Office Action fails to mention the proximate sections in Hoon et al. explaining that the cited comments are directed to breast cancer. Logically, Hoon et al. only provides teachings directed to the particular combinations contained in that patent and is deficient with respect to PAX3. Moreover, teachings directed to sensitivity in breast cancer do not predict behavior in melanoma. The evidence provided below establishes that the use of markers in making prognosis in melanoma (and other cancers) is replete with uncertainty.

The article *Prospective Multi-Institutional Study of Reverse Transcriptase Polymerase Chain Reaction for Molecular Staging of Melanoma* by C. R. Scoggins et al., (Journal of Clinical Oncology, Vol. 24, No. 18, p. 2849-2857 June 2006) investigates the use of tyrosinase, MART1, MAGE3, and GP-100 markers in staging melanoma (Attached as Exhibit B). Scoggins et al. provides the following commentary regarding the failures of melanoma marker analysis in providing a diagnostic advantage:

> In this study, RT-PCR analysis of PBMCs demonstrated that the prognosis of patients with one marker was no different than for those who had no markers detected. Expression of more than one marker was associated with worse DFS and DDFS; OS was not different.
>
> In conclusion, using a large, multicenter, randomized, prospective study, we did not find any predictive value of SLN RT-PCR analysis. Detection of more than one marker in PBMC was associated with worse prognosis, although the clinical utility of this assay is likely limited.
>
> Scoggins et al., p. 2855 (Emphasis added)

Scoggins et al. demonstrates a number of considerations that are relevant to the present invention. For example, the utility of melanoma markers was still uncertain a number of years after the filing of the present invention. In particular, with respect to the combination of tyrosinase, MART1, MAGE3, and GP-100, even the simultaneous expression of two markers was not associated with any longer overall survival than one or no marker expression. At the very least, this is support for the notion that "not all markers are created equal" and that the combination of the GalNacT and PAX3 markers discovered by the present invention is special with respect to predicting melanoma recurrence.

The article *Quantitative real-time PCR: a powerful ally in cancer research* by S. Mocellin et al. (TRENDS in Molecular Medicine, Vol.9, No.5, p. 189 – 195, May 2003) provides an assessment of the role of qRT-PCR in cancer research at the timeframe the present patent application was filed. (Attached as Exhibit C). The Examiner's argument fails to appreciate the role that qRT-PCR played in cancer research prior to the filing of the present patent application. The Mocellin article explains that qRT-PCR was used for detection of minimal residual disease, tumor immunology, DNA copy-number measurement, and genomic mutation and polymorphism. In particular, with respect to prognostic value, the Mocellin et al. article explains:

> Although the clinical utility of PCR-based MRD evaluation for hematological malignancies is well established, the experience

> with solid tumors is more limited. **Although some investigators have reported on the prognostic value of solid tumor [minimal residual disease] MRD detection at the molecular level [37], there is no general consensus on its clinical significance** [38]. Unlike hematological malignancies, solid tumors are rarely characterized by specific chromosomal translocations, and **tumor-specific markers are only expressed by some tumor types and in a relatively low percentage of cases**.
>
> Mocellin et al., p. 192 (emphasis added)

The Mocellin et al. article clearly eviscerates the Examiner's contention that quantification of cancer related markers can be correlated with prognosis. This proposition is simply not supported by the prevailing knowledge in the state of the art at the time the present patent application was filed.

The deficiency of the prior art is further supported by the article *A Meta-analysis of Reverse Transcriptase–Polymerase Chain Reaction for Tyrosinase mRNA as a Marker for Circulating Tumor Cells in Cutaneous Melanoma* by H. Tsao et al. (ARCH DERMATOL/VOL 137, MAR 2001 p. 325-330) which describes an experiment relating to the prognostic value of the tyrosinase mRNA marker in melanoma patients. (Attached as Exhibit D). The Tsao article explains the lack of such utility:

> Conclusions: The lack of data on the outcome of stage I, II, and III patients who were RT-PCR positive and **the low prevalence of RT-PCR positivity in patients with known stage IV disease limit the applicability of this test at this time**. Ongoing and future studies on a quantitative RT-PCR, amplification of multiple melanoma associated antigens, and use of the test as a prognostic indicator might improve the utility of this molecular serologic tool.
>
> Tsao et al. p. 325 (Emphasis added)
>
> Moreover, **the usefulness** and cost effectiveness of RT-PCR relative to other emerging serologic markers for melanoma, such as circulating S100 protein, 41-43 **remains to be established**.

Tsao et al. p. 325 (Emphasis added)

The Tsao article thus undercuts two assumptions by the Examiner. The Examiner's assertion that more markers are better is undercut by the confused state of the art circa 2002. Moreover, the utility of qRT-PCR and quantification of mRNA in general was unproven at that time.

The article *Simultaneous Immunohistochemical Detection of Tumor Cells in Lymph Nodes and Bone Marrow Aspirates in Breast Cancer and Its Correlation With Other Prognostic Factors* by B. Gerber et al. (Journal of Clinical Oncology, Vol 19, No 4 (February 15), 2001: pp 960-971) also casts considerable doubt on the recognized utility of qRT-PCR in providing prognostic value circa 2001. (Attached as Exhibit E). This article states with respect to breast cancer:

> Moreover, RT-PCR analysis is less expensive than currently available histopathologic examination techniques,[78] but it fails to distinguish benign from malignant epithelial cells.[67] Some cytokeratins (e.g., CK-19) **seem to have no diagnostic value as mRNA markers for micrometastases**; they are also expressed in blood and lymph nodes of healthy controls.
>
> Gerber et al., p. 968 (Emphasis added)

Many recent articles have shown qRT-PCR does not generally predict disease outcome in melanoma or other solid tumors. Moreover, prediction of disease outcome is useless unless a comparison to known prognostic factors for the tumor in question is performed in a multivariate statistical analysis as set forth in the present application. This proposition is supported in a recent article, *Molecular Staging of Pathologically Negative Sentinel Lymph Nodes from Melanoma Patients Using Multimarker, Quantitative Real-time rt-PCR.* by J.M. Hilari et al. (Ann Surg Oncol. 2009 Jan;16(1):177-85), which concludes that "multimarker qRT-PCR analysis of SLNs did not correlate with disease recurrence. Our data support specific

PAX3 splice variants but not GalNAc-T, PLAB or L1CAM as possible markers for melanoma metastasis to SLNs." (Attached as Exhibit F).

Another recent article, *Detection of Tyrosinase mRNA in the Sentinel Lymph Nodes of Melanoma Patients is Not a Predictor of Short-term Disease Recurrence* by C. Tatlidil et al. (Modern Pathology (2007), 20, 427-434), demonstrates that tyrosinase markers are of no prognostic value - "detection of tyrosinase mRNA by RT–PCR alone does not appear to increase the likelihood of short-term disease recurrence." (Attached as Exhibit G). Another article demonstrating that RT-PCR of a single marker is of minimal prognostic value is *Sentinel Lymph Node: Detection of Micrometastases of Melanoma in a Molecular Study* by VC Denninghoff et al. (Mol. Diagn. 2004; 8(4), (Abstract) 1 page) which observes that "[a]fter long follow-up period, molecular upstaging by tyrosinase RT-PCR failed to detect a subgroup of patients with an increased probability of recurrence." (Abstract attached as Exhibit H). Clearly, mRNA by RT-PCR alone does not appear to increase the likelihood of short-term disease recurrence. (See also *Prognostic Significance of Molecular Staging Study of Sentinel Lymph Nodes by Reverse Transcriptase-polymerase Chain Reaction for Tyrosinase in Melanoma Patients* by C. Mangas et al. (Annals of Surgical Oncology 13(7): 910-918). (Attached as Exhibit I).

The Scoggins, Mocellin, Tsao, Gerber, and more recent articles indicate that the relevancy of gene markers in cancer diagnosis was uncertain at the time the present patent application was filed. The references demonstrate that not just any combination of cancer related gene markers would be useful for determining a melanoma patient's prognosis. The Federal Circuit recently explained:

> To differentiate between proper and improper applications of "obvious to try," this court outlined two classes of situations where "obvious to try" is erroneously equated with obviousness under § 103. In the first class of cases,

> what would have been "obvious to try" would have been to vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful.
>
> *Id.*

> In such circumstances, where a defendant merely throws metaphorical darts at a board filled with combinatorial prior art possibilities, courts should not succumb to hindsight claims of obviousness.
>
> *In re Kubin,* (Fed. Cir. 2009)

The prior art in the context of the present invention exemplifies a situation in which there are a number of possible choices without an indication of which parameters are critical. In such a multifaceted disease such as cancer, blanket conclusions regarding the types of cancers, cancer gene markers, the expression levels of such gene markers, and the correlation of prognosis therewith are not obvious. This uncertainty is clearly realized by the Mocellin article's observation that "[a]lthough the clinical utility of PCR-based MRD evaluation for hematological malignancies is well established, the experience with solid tumors is more limited." Clearly, conclusions regarding hematological cancers cannot be imputed to solid tumors. The present invention provides a novel predicative method in which the levels of a plurality of mRNA transcripts have been found useful for evaluating the prognosis of a cancer patient – a melanoma patient.

Accordingly, claims 1-3, 5-7, 10, 34-36, and 38-40 are patentable under 35 U.S.C. § 103(a) over Hoon et al. in view of Scholl et al.

Claims 1-7, 10, 34-40 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Hoon et al. (U.S. Patent 6,057,105; 5/2/00) in view of Scholl et al. (2/01, Cancer Research, 61:823-826) as applied to claims 1-3, 5-7, 10, 34-36, and 38-40 above, and further in view of Johansson et al. (2000, Clinical Chemistry, 46(7): 921-927).

Claim 6 is incorporated into claim 1.

The deficiencies of Hoon et al. with respect to using PAX3 to detect metastatic melanoma in histopathologically negative lymph nodes and the non-obviousness of the combination PAX3 and GalNacT are set forth above. Johansson does not cure these defects of Hoon et al. nor Scholl et al. and is not used for that purpose. The Office Action only relies on Johansson to teach "a reproducible method comprising performing qRT-PCR to quantitatively detect mRNA markers of melanoma in biological samples" (Office Action, p. 9). This reliance is irrelevant because the combination of markers set forth in each of the independent claims is novel and non-obvious as set forth above.

Accordingly, for at least these reasons, claims 1 and 34, along with dependent claims 2-7 and 10, are patentable over the combination of Hoon, Scholl and Johansson, and Applicant respectfully requests reconsideration and withdrawal of this rejection under 35 U.S.C. 103(a).

Conclusion

Applicants have responded to each of the Examiner's objections and rejections to advance the prosecution of this case. Applicants believe that all formal and substantive requirements for patentability have been met and that this case is in condition for allowance, which action is respectfully requested. If any additional issues need to be resolved, the Examiner is invited to contact the undersigned at his earliest convenience.

The Petition fee of $245.00 is being charged to Deposit Account No. 02-3978 via electronic authorization submitted concurrently herewith. The Commissioner is hereby authorized to charge any additional fees or credit any overpayments as a result of the filing of this paper to Deposit Account No. 02-3978.

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Respectfully submitted,

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